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A comparison of screening methods for insect contamination in wheat

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Abstract

In collaboration with the United States Department of Agriculture and a number of major milling companies, the “Insect-Detect” immunoassay for analyzing insect contamination in grains has been compared with three more traditional methods, X-ray analysis, cracking and flotation, and the insect fragment test (IFT). Testing was carried out in blind fashion using clean wheat samples that were spiked with differing numbers of grain kernels infested with late instar larvae of the granary weevil (*Sitophilus granarius* (L.)). Three different laboratories analyzed the samples for each of the four methods. The collaborative trials showed that the insect immunoassay clearly provided the most accurate measurement of actual insect infestation, followed by X-ray analysis. While both cracking and flotation and IFT procedures provided a general measure of contamination, they showed much greater variability. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Immunoassay; Insect infestation in wheat; X-ray; Cracking and flotation; Insect fragment test

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1. Introduction

Cereal crops are major sources of foods for human consumption in many parts of world, including the United States. Managing stored grain wisely with minimal loss while maintaining its nutritional quality is a major task. Postharvest food losses are estimated to range from 9% in the United States (Pimentel, 1991) up to 50% in some parts of developing nations. Much of the loss results from the invasion of the grain mass by mold, insects and rodent pests. In the US alone, the grain loss due to insect damage is estimated to be around 1 billion dollars per year (Oklahoma Cooperative Extension Service, 1995). Many species of insects have found stored grains to be a favorable environment and they reproduce more or less continuously under proper temperature and moisture conditions.

In the US, the Food and Drug Administration (FDA) has set the defect action level (DAL) as the regulatory standard for quality control. For insect contamination, the DAL is 32 insect damaged kernels per 100 g of wheat and 75 insect fragments per 50 g of wheat flour (FDA, 1998). Millers routinely screen their grain to determine whether they meet requirements of the law and to ensure their flour quality. The most commonly used methods for determining insect contamination are a visual test for insect damaged kernels (IDK) (Russell, 1988) and the acid hydrolysis test (AACC method 28–41A or one of its variants) (AACC, 1995), commonly referred to as the insect fragment test (IFT). The IDK method involves visually inspecting and counting the number of damaged kernels in 100 g of wheat and reporting the number of damaged kernels. The IFT involves milling, extracting, and counting microscopically the number of insect fragments produced during the milling procedure. Two other grain screening tests used currently are an X-ray method (AACC method 28–21) and the cracking and flotation method (AACC methods 28–22 and 28–51) (AACC, 1995). Recently, a new grain screening tool, a sandwich enzyme-linked immunosorbent assay (ELISA) for insect detection, became available. This method can measure insect contamination with great precision and speed (Kitto, 1991). While the first four methods mentioned above have been in use for many years, little work has been published on how the different methods compare with one another or how well any of the methods predict the number of insect fragments in flour. In this paper, efforts were made to compare the accuracy of these methods, including the immunoassay, for determination of actual hidden insect contamination.

The plan that evolved entailed testing the various methods under comparable conditions. The samples were prepared in one location and tested in a blind manner by different collaborators. The levels of contamination, the species of the infesting insect, and the developmental stage of the insect in all samples were carefully controlled. *Sitophilus granarius* (L.) (granary weevil) is one of the major grain-infesting insects and was used in this study because of its ability to internally infest grain. The larvae of *S. granarius* live entirely within the kernel, where they feed unseen and usually unsuspected. Each adult female can lay up to 250 eggs during its lifetime (ARS, 1986). This kind of infestation is a major concern for the grain industry because the larvae cannot be removed by ordinary machinery during cleaning and are a major source of insect contamination in grain products. The following methods were compared in this study: the IFT, cracking and flotation, X-ray analysis, and the immunoassay. We did not include the IDK method because it was found to be unreliable for detecting internal infestation by weevils, as reported by Russell (1988).

2. Materials and methods

2.1. Sample preparation

The USDA/Agricultural Research Service (ARS) Laboratory at the University of Wisconsin prepared the infested kernels. Soft, white winter wheat was used as the grain and *S. granarius* was used as the infesting insect. Several hundred kernels were infested by placing two mated *S. granarius* females together with two kernels of soft wheat and small amounts of cracked wheat in glass vials. The weevils fed on the cracked wheat while using the intact kernels for oviposition. Females and cracked wheat were removed after 2 days and the infested kernels were separated and individually kept in glass vials. Richards and Oxley (1943, cited in Longstaff, 1981) had reported that late instar larvae of *S. granarius* very often and especially under low carbon dioxide conditions make small holes into the grain and eject much of the frass. Therefore, the larvae of *S. granarius* inside the kernels were allowed to develop, at room temperature, to a late larval instar as indicated by the time span of development and by small amounts of visible frass in each vial before freezing them to stop further development. Sample sets were made up by spiking clean grains with various numbers of infested kernels. Each sample contained from 0 to 12 infested kernels per 50 g. After all samples were prepared, the remaining infested kernels were dissected. All of them were found to contain late instar larvae. The USDA/ARS Laboratory kept the record of the sample codes for each sample set and then sent sample sets to the participating laboratories.

2.2. Sample distribution to participants

A protocol was devised among participants that included the following: (1) Each laboratory would assay one set of eight samples (50 g/sample). (2) All sample sets would contain samples with the same levels of insect contamination. (3) Laboratories must routinely use the method assigned. (4) Each laboratory would run its set of samples blind, without knowing the exact numbers of infested kernels added. (5) For each of the four methods, three different laboratories would each analyze a set of samples. A total of 11 laboratories participated in the study, with one of them capable of doing IFT and ELISA to test two separate sample sets.

2.3. Insect fragment test (AACC method 28-41A)

This method is also known as the Acid Hydrolysis method and is the method (or one of its variants) used by the FDA to define the DAL for insect contamination in flour. All three laboratories that tested the comparison samples use this method routinely. In brief, a 50 g milled grain sample was mixed with 500 ml of 5% HCl. Light mineral oil was added. The sample was boiled for 10 min with stirring and transferred to a separation funnel. The lower layer was drained off to about 2.5 cm of interface. The sample was then washed with hot tap water. The separation, draining and washing steps were repeated twice. After the final wash, the sample was filtered through a lined filter paper. The filter paper was examined under a microscope and the number of insect fragments was counted.

Some participating laboratories were not able to mill small quantities of grain samples. A master set of eight 250 g spiked samples were, therefore, prepared in the USDA/ARS Laboratory

and milled by the USDA/grain inspection, packers and stockyards administration (GIPSA) Laboratories at Kansas City, MO, to preserve the limited supply of infested kernels. Each of the 250 g samples contained from 0 to 60 infested kernels. The USDA/GIPSA kept one set of eight 50 g sub-samples from each level of infestation and sent other sets to the participating laboratories.

2.4. Cracking and flotation method (AACC methods 28–22 and 28–51)

There are several different ways to do the cracking and flotation test. In general, grain samples were screened using a No. 12 sieve (1.7 mm opening), ground, and put in a trap flask. Isopropyl alcohol (60%) saturated with heptane was thoroughly mixed with the sample. Then the sample was washed down the sides of the flask with isopropyl alcohol until about 400 ml was added and soaked for 30 min. The material at the top of the flask was trapped off twice, using 20–30 ml of heptane for each trapping. The trappings were filtered on lined filter paper or 10 × bolting cloth and examined under a microscope.

2.5. X-ray analysis (AACC method 28–21)

Grain samples were brushed for a short period over a 13–20 cm No. 12 screen, using a stiff-bristled brush to remove any surface insects, dust, dirt or broken kernels through the screen as completely as possible. The kernels were then spread and radiographed using the manufacturer's recommendation for film development. The radiograph was then examined for insect-damaged grains. Radiographic illustrations of insect-infested kernels can be found in AACC method 28–95.

2.6. Immunoassay method

The sandwich ELISA for detection of insects uses antibodies that can specifically bind to the insect muscle protein, myosin, which is common to all insects (Quinn et al., 1992). The sample preparation involved grinding a 50 g grain sample and extracting it with 100 ml phosphate buffer in a common household blender. The extract was then centrifuged and tested by pipetting 50 ml of supernatant into an antibody-coated microwell strip and incubating for 18 min. After washing with buffer, a subsequent series of reactions requiring 25 min produces a color in the wells whose intensity is proportional to the number of insects present in the sample. The absorbency of the color at 414 nm was measured using a plate reader (Multiskan Plus, Labsystems, Needham Heights, MA or one of its equivalents). A series of dilutions of myosin used as the standard and buffer as the blank were run simultaneously with the samples.

2.7. Statistical methods for result comparison

Linear regression analyses were performed on all sets of data. A best fitting straight line, $Y = A + BX$, with least square was generated for each data set (Brown and Hollander, 1977). X values on the horizontal axis are the numbers of infested kernels added to each sample and Y values on the vertical axis are the outputs of four testing methods. A is the intercept and B is the slope. The standard deviation (SD), R^2 and P were also calculated and used to compare all four

methods under study and between the participating laboratories. SD is the standard deviation of the vertical distances of the points from the line. R^2 is the fraction of the variation that is shared between X and Y . It is a value between 0 and 1. When R^2 equals 0, there is no linear relationship between X and Y . When $R^2 = 1$, all points lie exactly on a straight line with no scatter. It is possible to have a R^2 value of more than zero when the true correlation is zero. The P value is for testing the null hypothesis that the slope is zero, and is the probability that randomly selected points would result in a regression line of $B > 0$ when there is no linear relationship between X and Y . The lower a P value, the better is the association between X and Y .

3. Results

3.1. Insect fragment test

The results of the fragment count from three different laboratories are given in Fig. 1. The number of added infested kernels per 50 g is plotted against the number of fragments observed. Results from Labs 1 and 2 showed a very poor correlation between the fragment counts and the number of added kernels while Lab 3 showed large fluctuations but with a trend that was generally related to an increasing number of fragments counted. Furthermore, there was a wide variation in results between laboratories measuring the same level of contamination. For instance, the fragment counts of samples with six infested kernels from the three participating laboratories were 15, 34, and 200, respectively. Also, of the 24 samples assayed only 3 samples were found with 75 or more fragments per 50 g, which is the DAL for wheat flour. One of these samples had only one infested kernel per 50 g while the other two had 6 and 12 infested kernels. For the samples

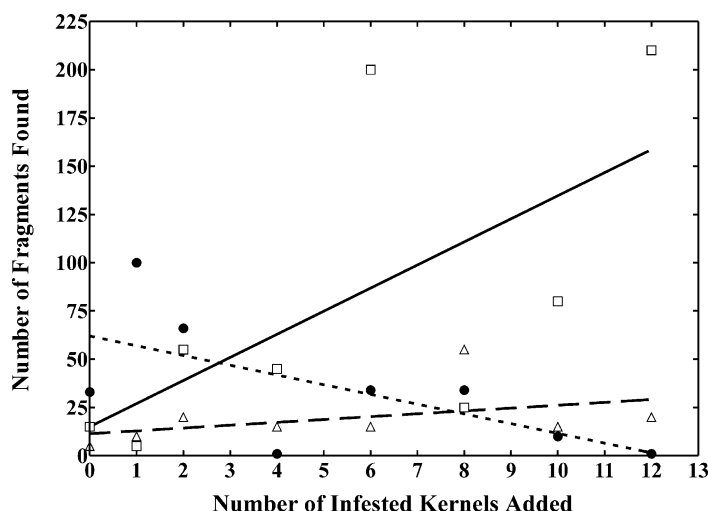


Fig. 1. Detection of insect contamination in wheat samples spiked with various levels of infested kernels using insect fragment test. Three separate laboratories carried out the assays and the results were analyzed using linear regression. Lab 1 — \triangle —, Lab 2 — \bullet —, Lab 3 — \square —.

with no infested kernels, 5, 33, and 15 fragments were found by the three laboratories, respectively, indicating the possibility of false positives. For the samples spiked with 12 infested kernels, Lab 2 found only one fragment, suggesting the probability of false negatives.

3.2. Cracking and flotation

Three laboratories assayed the samples using the cracking and flotation method. The results are given in Fig. 2a–c. The results from Lab 4 (Fig. 2a) and Lab 6 (Fig. 2c) indicate that there is a fairly good correlation between the counts from the cracking and flotation method and the number of infested kernels in the sample. However, data from Lab 5 (Fig. 2b) shows a rather poor correlation. Although the AACC method 28–22 requires counting whole insects, cast skins and head capsules when the samples are examined microscopically, Labs 4, 5, and 6 involved in this project counted the number of total fragments, the number of heads and larvae, and the number of mandibles, respectively. These designations should result in roughly similar results because only

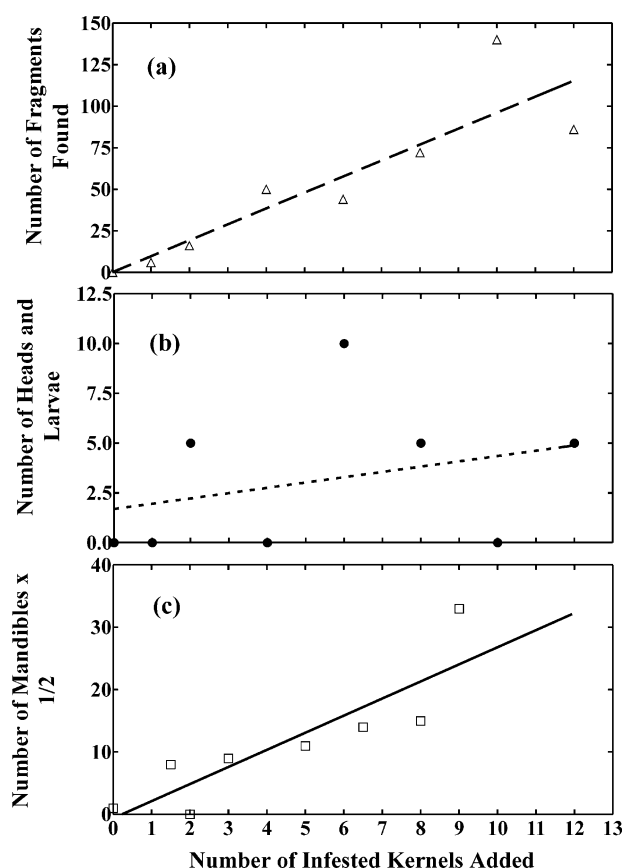


Fig. 2. Detection of insect contamination in wheat samples spiked with various levels of infested kernels using cracking and flotation. Three separate laboratories carried out the assays and the results were analyzed using linear regression. (a) Lab 4 —△—, (b) Lab 5 —●—, (c) Lab 6 —□—.

the mandibles of the larvae are highly sclerotized. Nevertheless, the numbers of total fragments found by Lab 4 range from 0 to 144 in all eight samples, while the Lab 6 found 0 to 33 mandibles and Lab 5 found 0 to 10 heads and larvae. The results for insect-free samples analyzed by all three labs are similar with 0, 0, and 1 fragment found. However, false-negatives are possible since zero fragments were found in samples with up to 10 infested kernels.

3.3. X-ray analysis

Results from Labs 7–9 using X-ray analysis are shown in Fig. 3. Lab 7 was an academic institution while Labs 8 and 9 were major milling company laboratories. The observed infested kernels in all samples are usually within ± 3 insects of the actual number. For samples with no infested kernel added, Lab 7 found zero contamination, but Labs 8 and 9 found 3 and 1 infested kernels, respectively. In general, results from Labs 7 and 9 show a good correlation between the numbers of infested kernels found by X-ray analysis and the numbers of infested kernels added in the samples. The results of Lab 8 showed less correlation.

3.4. Immunoassay

In laboratory trials the immunoassay method gives very reproducible standard curves. For example, Fig. 4 illustrates typical assay results when adult *S. granarius* are added to insect-free wheat. In the assay, the color change from no infestation to 10 *S. granarius*/50 g is steep, allowing for a quantitative estimate of the number of infesting insects, i.e., adult *S. granarius* equivalents, by interpolation of the color produced by an unknown sample. Fig. 5 is a plot of the results obtained by the three laboratories participating in the current comparative trial. All three laboratories detected zero weevils in the samples with no infested kernels. No false-negatives were

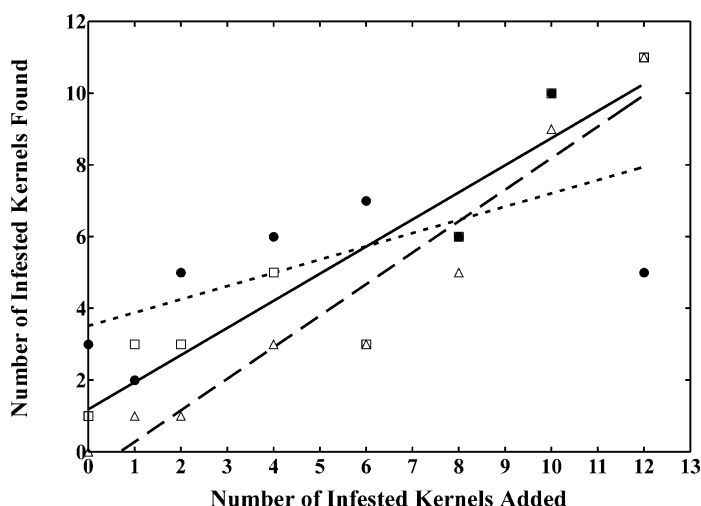


Fig. 3. Detection of insect contamination in wheat samples spiked with various levels of infested kernels using X-ray. Three separate laboratories carried out the assays and the results were analyzed using linear regression. Lab 7 —△—, Lab 8 ----●----, Lab 9 —□—.

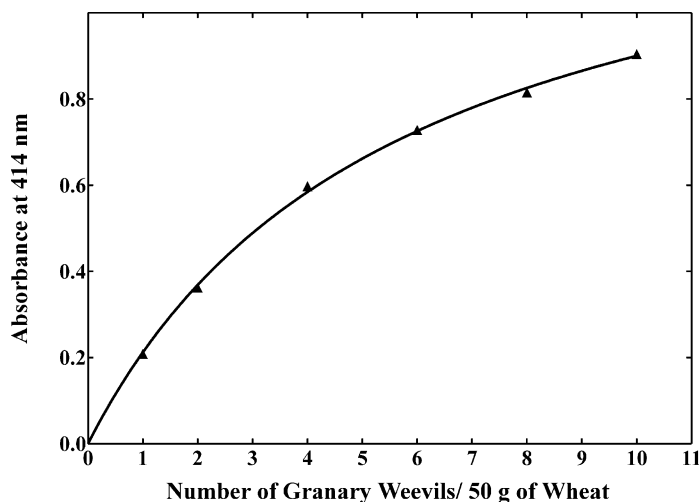


Fig. 4. A typical standard curve for analysis of granary weevils in wheat samples using ELISA.

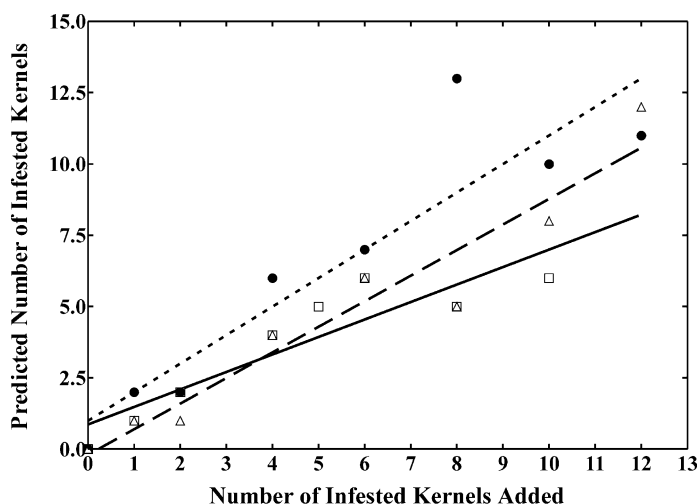


Fig. 5. Detection of insect contamination in wheat samples spiked with various levels of infested kernels using enzyme-linked immunosorbent assay (ELISA). Three separate laboratories carried out the assays and the results were analyzed using linear regression. Lab 10 — \triangle —, Lab 1 ---- \bullet ----, Lab 11 — \square —.

found, either. The predicted values for samples with fewer than six infested kernels were good and usually within ± 1 insect of the actual number. When the number of infested kernels in the samples is higher than 6, the accuracy of prediction by the immunoassay seems to decrease, as shown by data from Lab 11. A change in protocol for Lab 11 was necessitated by the loss of the original sample set during transit. Because the USDA/ARS had no more infested kernels needed to prepare a duplicate set, a set of samples was prepared at the Biotect laboratories by adding 0, 1, 2, 4, 5, 6, 8, and 10 adult granary weevils to each 50 g wheat sample and then sent to Lab 11.

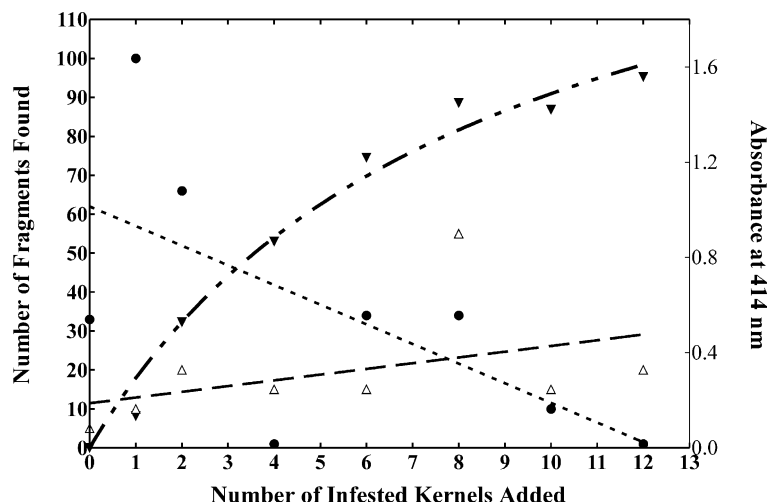


Fig. 6. A direct comparison of the immunoassay and insect fragment test. One sub-sample set from a master sample set was analyzed using immunoassay (—▼—), and two sub-sample sets from the same master sample set were analyzed using insect fragment test (---●---, ---△---).

A direct comparison of the results from immunoassay and IFT can be made using the data from the master sample set prepared and milled at the USDA/GIPSA. One set of samples remaining from the fragment count comparisons was analyzed using the immunoassay procedure and compared with two sets of results from the IFT method. The results for each method on the same samples are shown in Fig. 6. The fragment counts show no general response to the increasing level of internal insect contamination. The immunoassay on the other hand shows an excellent correspondence up to a contamination level of six added weevils. Beyond six weevils the color response flattened, making predictions of contamination less precise.

4. Discussion

The methods used in this comparative study for testing of insects were the IFT, cracking and flotation, X-ray analysis, and immunoassays. The first three assays were carried out using AACC approved methods; the immunoassay method is not yet approved. Results from all tests are consolidated in Table 1 where the total number of samples tested in each data set (N), A , B , SD , R^2 and P values are listed by method as well as by individual laboratory. In general, IFT was the least accurate method used in this collaborative study for predicting the insect contamination in wheat. The result from Lab 1 has a R^2 of 0.178 and P of 0.297, suggesting a lack of an association between the fragment count and the number of infested kernels. Lab 2 data has a negative B value, a R^2 of 0.419 and a P of 0.0825, indicating a weak, negative association between the fragment counts and the level of insect contamination in the samples. The result from Lab 3 is the only one which shows a weak, positive correlation with a R^2 of 0.416 and a P of 0.0840. As for cracking and flotation, two labs (Labs 4 and 6) obtained similar, fairly good correlations between

Table 1
Comparison of the four methods using linear regression analysis

Method	Lab. no.	<i>N</i>	<i>A</i>	<i>B</i>	SD	<i>R</i> ²	<i>P</i>
IFT	1	8	11.470 ± 8.699	1.471 ± 1.288	14.901	0.178	0.2970
	2	8	62.001 ± 16.372	−5.047 ± 2.424	28.045	0.419	0.0825
	3	8	15.061 ± 39.005	11.965 ± 5.782	66.901	0.416	0.0840
C&F ^a	4	8	−0.168 ± 13.385	9.597 ± 1.981	22.928	0.796	0.0029
	5	8	1.695 ± 2.228	0.266 ± 0.330	3.816	0.098	0.4506
	6	8	−0.618 ± 3.415	2.741 ± 0.640	5.523	0.753	0.0052
X-ray	7	8	−0.598 ± 0.654	0.879 ± 0.097	1.120	0.932	0.0001
	8	8	3.512 ± 1.160	0.370 ± 0.172	1.987	0.436	0.0748
	9	8	1.185 ± 0.857	0.756 ± 0.127	1.468	0.856	0.0010
ELISA	10	8	−0.198 ± 0.678	0.897 ± 0.100	1.161	0.930	0.0001
	1	8	1.0 ± 1.167	1.0 ± 0.173	2	0.847	0.0012
	11	8	0.866 ± 0.620	0.613 ± 0.112	1.025	0.834	0.0015

^aCracking and flotation.

the numbers of fragments found and the numbers of infested kernels added, with *R*² of 0.796 and 0.753. However, the result from Lab 5 has a high *P* of 0.4506 and a low *R*² of 0.098, indicating no association. X-ray analysis is another example of high variability between different laboratories. Labs 7 and 9 obtained good correlation with *R*² of 0.932 and 0.856, respectively. But Lab 8 gave results of similar accuracy as the IFT, with a *R*² of 0.436 and a *P* of 0.0748. For immunoassay, the high *R*² values, low *P* values and good consistency among all three labs indicate that this method provided a highly accurate, quantitative measure for the number of infested kernels present in the sample mix.

The immunoassay method was compared directly with IFT on a common sample blend. This comparison showed that the poor results from the IFT were not due to inadequate blending of the milled master samples. It also indicated that the immunoassay can give a much more reliable prediction of hidden contamination than the IFT.

The IFT and visual examination for IDK are the two methods currently used most often by the industry. Among the methods tested in this study, the IFT was shown to be the least reliable method for predicting insect infested kernels in wheat. The low *R*² values from the IFT method were indicative of there being no significant correlation between insect fragment counts and actual insect infestation. This finding is consistent with an earlier study of Kurtz (1965), which indicated that IFT is incapable of giving reproducible results. As for the visual examination for IDK, Russell (1988) reported that the method was unable to predict hidden insect infestation in wheat grain; in fact, the presence of damaged kernels should be interpreted as evidence of a potentially serious infestation problem. The X-ray method and cracking and flotation are relatively good in estimating internal infestation as observed in the current study and reported by Russell (1988). However, the X-ray method necessitates the use of expensive equipment, and the cracking and flotation method requires the use and disposal of hazardous organic solvents; therefore, the immunoassay is clearly the preferable, reliable method.

With varying degrees of precision, the immunoassay, X-ray, and cracking and flotation methods measure insect contamination in grain. The collaborative trials demonstrated that, of the methods compared, immunoassays are more precise and more consistent from lab to lab than the other methods. Furthermore, the time needed for extracting and assaying one sample using the immunoassay is less than 1 h. By contrast, it would take about 2.5, 8 and 1.5 h to finish the analysis of one sample using X-ray, IFT, and cracking and flotation, respectively. Testing of multiple samples at the same time can surely shorten the average assay time per sample. Nevertheless, immunoassay is still definitely the fastest among all four methods with an average of 7 min per sample when assaying eight samples simultaneously.

Importantly, while the X-ray and cracking and flotation methods are relatively reliable for measuring actual insect contamination in grain, they cannot be used to assay flour because the integrity of the kernel is destroyed when the grain is milled. Neither X-ray nor cracking and flotation methods can predict the number of insect fragments in the flour. Also, the IFT when carried out on ground grain samples does not predict the number of fragments that will be produced when grain is milled. The large experimental error inherent with the method is great enough to make it impossible to have reproducible results, and the number of fragments observed will vary according to the stage of insect development, and whether the insects are alive or dead and how long dead (Sachdeva, 1978).

By contrast, immunoassays can be used to assay both grain and flour. Studies at Biotect and The University of Texas have determined that immunoassays may be used to analyze flour if the assay is carried out immediately after milling or if flour samples are frozen soon after milling and kept frozen. The correlation between immunoassays carried out on grain and on milled flour is excellent (Kitto, 1991). The immunoassay is also capable of detecting a wide variety of common pests of stored grain and the assay response is proportional to the weight of each insect (Quinn, 1991). Although the immunoassay is not able to determine which species of the insects are infesting the grain samples, it can perfectly serve as a screening method due to its superior accuracy and speed. Species-specific immunoassays are possible solutions for this problem and an immunoassay specific to *S. granarius* has been developed (Chen and Kitto, 1993).

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References

- ARS—Agriculture Research Service, 1986. Stored-Grain Insects. Agriculture Handbook No. 500. United States Department of Agriculture, Washington, DC, 57pp.

- AACC—American Association of Cereal Chemists, 1995. Acid hydrolysis method for insect fragments and rodent hairs-flour, AACC method 28–41A. In: Approved Methods of the American Association of Cereal Chemists, 9th Edition. ACC, St Paul, MN.
- Brown, B.W., Hollander, M., 1977. Linear regression and correlation. In: Brown, B.W., Hollander, M. (Eds.), Statistics: A Biomedical Introduction. Wiley, New York, NY, pp. 261–308.
- Chen, W.M., Kitto, G.B., 1993. Species-specific immunoassay for *Sitophilus granarius* in wheat. Food and Agricultural Immunology 5, 165–175.
- FDA—Food and Drug Administration, Center for Food Safety and Nutrition, 1998. The Food Defect Action Levels, Washington, DC.
- Kitto, G.B., 1991. A new rapid biochemical technique for quantitating insect contamination in grain. Association of Operative Millers Technical Bulletin, March, 5835–5838.
- Kurtz, O.D., 1965. Comparison of AACC and AOAC methods for extraneous materials in flour. Journal of the Association of Official Analytical Chemists 48, 554.
- Longstaff, B.C., 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. Protection Ecology 2, 83–130.
- Oklahoma Cooperative Extension Service, 1995. Stored Products Management. Circular No. E-912 (Chapter 32).
- Pimentel, D., 1991. World resources and food losses to pests. In: Gorham, J.R. (Ed.), Ecology and Management of Food Industry Pests, FDA Technical Bulletin 4. Association of Official Analytical Chemists, Gaithersburg, MD, pp. 5–11.
- Quinn, F.A., 1991. Development of enzyme immunoassay techniques for detecting insect antigens in wheat. Dissertation. The University of Texas, Austin, TX.
- Quinn, F.A., Burkholder, W., Kitto, G.B., 1992. Immunological technique for measuring insect contamination of grain. Journal of Economic Entomology 85, 1463–1470.
- Russell, G.E., 1988. Evaluation of four analytical methods to detect weevils in wheat: Granary weevil, *Sitophilus granarius* (L.), in soft white wheat. Journal of Food Protection 51, 547–553.
- Sachdeva, A.S., 1978. Effect of infestation stage, form and treatment on fragment count in flour. M.S. Thesis. Kansas State University, Manhattan, KS.